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## Interview Summary

Application No.  
08/898,736

Applicant(s)  
Coppens et al

Examiner  
Curtis E. Sherrer

Group Art Unit  
1761

All participants (applicant, applicant's representative, PTO personnel):

(1) Curtis E. Sherrer (3) \_\_\_\_\_

(2) Timothy E. Levstik (4) \_\_\_\_\_

Date of Interview Aug 24, 2000

Type: ☐ Telephonic ☒ Personal (copy is given to ☐ applicant ☒ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No. If yes, brief description:

Agreement ☐ was reached. ☒ was not reached.

Claim(s) discussed: All claims in general

Identification of prior art discussed:

Gyllang et al (EBC, 16th Congress)

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:

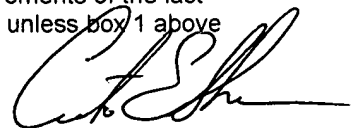
Discussed the Draft Supplemental Declaration of Coppens (see attached), which provides more detail as to how the previously performed experiments were performed. It was suggested that Applicants submit the actual sizes of the spores in the pictures and that the pictures be related to the Gyllang experiment. It was noted, from the Declaration, page 8, par. 7, that the "successful activation depends on incubation of dormant spores for a sufficient time at a suitable temperature and in a suitable medium" and therefore some of the claims, e.g. Claim 53, may not be enabled.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.



CURTIS E. SHERRER  
PRIMARY EXAMINER  
ART UNIT 1761

Examiner Note: You must sign and stamp this form unless it is an attachment to a signed Office action.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Coppens et al. )  
Serial No.: 08/898,736 )  
Filed: July 23, 1997 )  
Title: PROCESS FOR THE )  
PREPARATION OF MALTED )  
CEREALS )  
Group Art Unit: 1761 )  
Examiner: C. Sherrer )

*For your 11 am interview  
Please see pgs 4-8*

SUPPLEMENTAL DECLARATION OF THEO COPPENS UNDER 37 CFR 1.132

Honorable Commissioner of Patents  
and Trademarks  
Washington, D.C. 20231

Dear Sir:

I, Theo Coppens, pursuant to 37 C.F.R. §1.132, declare as follows:

1. I am one of the inventors for the above-identified patent application.

2. In 1999, I asked Prof. C. Michiels, Professor of the Faculty of Agricultural and Applied Biological Sciences at Katholieke Universiteit Leuven in Belgium, to conduct the following experiments under my supervision to determine whether the medium and growth conditions described in Gyllang et al. would provide activated spores. Those experiments and their results were first reported in my Declaration signed on

July 9, 1999. A more detailed explanation of those experiments and their results is presented herewith.

### Materials and Methods

3. Chemicals and Media. Peptone, Yeast Extract and Potato Dextrose Agar (PDA) were obtained from Unipath (Hampshire, United Kingdom). Dextrose was obtained from Merck-Belgolabo (Leuven, Belgium). Peptone, yeast extract and dextrose medium were prepared according to Kaiser et al. (1994). Peptone (2% w/v), yeast extract (1% w/v) and dextrose (2% w/v) were dissolved in deionised water. The medium was sterilized at 121°C for 15 minutes. The pH of the obtained medium was 6.4.

4. Fungal Strains: Cultivation and Preparation of Culture Homogenate. The strains *Rhizopus oryzae* ATCC 9363, *Aspergillus fumigatus* CBS 148.89 and *Aspergillus amstelodami* VTTD-76035 were obtained from respectively the American Type Culture Collection (ATCC, Manassas, VA, USA), Centraalbureau voor Schimmelcultures (CBS, Baarn, The Netherlands) and VTT (Technical Research Centre of Finland, Espoo, Finland) Culture collections. The strains were grown on PDA at 28°C. Seven days old sporulating cultures on PDA served as the starting material for culturing the fungi as described by Gyllang et al. (1977). For each strain, a loopful of material taken from the seven-day old sporulating culture on PDA was inoculated in a tissue culture flask containing 225 ml of Peptone, Yeast Extract and Dextrose medium. The culture was grown for 3 weeks at 20°C. After the cultivation period the entire culture was homogenized by vigorously shaking the content of the tissue culture flask.

5. Analysis of activation of the spores in the culture homogenate. Activated spores were defined as described in the current patent application as "being significantly more swollen than the dormant size, the size of the spores being increased by a factor preferably between 1.2 and 10 over the dormant spore size and/or having one or more germ tubes per spore."

One of the first steps of activation is, indeed, uptake of water, and this is reflected by an increase in volume of the spore by swelling and/or by formation of one or more germ tubes.

In order to detect spore activation, we selected a multiplier increase of 1.2 over the high end of the dormant spore size of the fungus under study (cfr. paragraph 6 hereunder). In other words, as spores of *Rhizopus oryzae* are typically in a size range of 5.0 to 8.0  $\mu\text{m}$  (cfr. paragraph 6), spores were considered to be activated only if their dimensions exceeded 8.0 x 1.2  $\mu\text{m}$  (the lower size range would have yielded 5 x 1.2  $\mu\text{m}$ , and this value is within the range of the sizes of the dormant spores).

Three different samples of 0.2 mL of the culture homogenate were examined microscopically, and at least 100 spores per sample were evaluated. Swelling of the spores was verified by evaluating the spore size at a magnification of 1250x by means of an eyepiece graticule micrometer. Also, the formation of germ tubes was evaluated microscopically. All observed spores were divided in to classes: those that are activated according to the above criteria and those that are not activated. Activation was calculated as an average of the three samples, and reported as a percentage of the spore population.

## Results

6. Analysis of spore activation. The dormant size of various fungal spores is described by Pitt and Hocking (1997). According to this reference, the sporangiospores of *Rhizopus oryzae* are of variable shape, ellipsoidal to broadly fusiform or irregularly angular, commonly 5.0-8.0  $\mu\text{m}$  long, the conidiospores of *Aspergillus amstelodami* are spherical to subspheroidal with 4.0-5.0  $\mu\text{m}$  diameter; the conidiospores of *Aspergillus fumigatus* are spherical to subspheroidal with 2.5-3.0  $\mu\text{m}$  diameter. Our own observations of dormant spores of the three tested strains were in agreement with the description given by Pitt and Hocking (1997). Accordingly, using a multiplier of 1.2 which we thought appropriate for these spore types and an unactivated spore size on the large end of the ranges as previously described, we defined activated spores of *Rhizopus oryzae* ATCC 9363, *Aspergillus fumigatus* CBS 148.89 and *Aspergillus amstelodami* VTT D-76035 as having respectively a size of more than 9.6  $\mu\text{m}$ , 6.0  $\mu\text{m}$  and 3.6  $\mu\text{m}$  and/or one or more germ tubes per spore. Figure 1 shows some microphotographs of dormant, swollen and activated spores of *Rhizopus oryzae* ATCC 9363.

Figure 1. Activated spores obtained by treatment as described in the current patent application (magnification 720x): A. dormant spores; B. swollen spores with one activated (Ac) spore, i.e. significantly more swollen than the dormant size; C. activated spores significantly more swollen than the dormant size and having one or more germ tubes per spore.

The results of the analysis of spore activation in the culture homogenates immediately after homogenization (0 time) under the procedure of Gyllang et al. (1977) are presented in Table I.

Table I

## Spore activation at 0 time in the culture homogenates

	sample number	No. of spores counted	No. of spores activated	No. of spores not activated	activation (%)	average activation (%)
<i>Rhizopus oryzae</i> ATCC 9363	1	120	0	120	0	0
	2	117	0	117	0	
	3	143	0	143	0	
<i>Aspergillus fumigatus</i> CBS 148.89	1	109	0	109	0	0
	2	115	0	115	0	
	3	103	0	103	0	
<i>Aspergillus amstelodami</i> VTT D-76035	1	156	0	156	0	0
	2	133	0	133	0	
	3	142	0	142	0	

Further, activation of spores in the culture homogenates was analyzed after 6 hours incubation of the culture homogenates at 20°C or 42°C, although this deviates from the procedure of Gyllang et al. (1977). In the latter, no incubation period and no temperature elevation is prescribed between preparation of the homogenate and inoculation of the barley. The results are presented in Table II.a and II.b.



Table II.a

Spore activation after 6 hours incubation of the culture  
homogenates at 20°C

	sample number	No. of spores counted	No. of spores activated	No. of spores not activated	activation (%)	average activation (%)
<i>Rhizopus oryzae</i> ATCC 9363	1	137	0	137	0	0
	2	109	0	109	0	
	3	125	0	125	0	
<i>Aspergillus fumigatus</i> CBS 148.89	1	101	0	101	0	0
	2	114	0	114	0	
	3	106	0	106	0	
<i>Aspergillus amstelodami</i> VTT D-76035	1	111	3	108	3	3
	2	127	5	122	4	
	3	108	2	106	2	

Table II.b

Spore activation after 6 hours incubation of the culture  
homogenates at 42°C

	sample number	No. of spores counted	No. of spores activated	No. of spores not activated	activation (%)	average activation (%)
<i>Rhizopus oryzae</i> ATCC 9363	1	122	0	122	0	0
	2	117	0	117	0	
	3	106	0	145	0	
<i>Aspergillus fumigatus</i> CBS 148.89	1	145	0	106	0	0
	2	129	0	129	0	
	3	128	0	128	0	

	sample number	No. of spores counted	No. of spores activated	No. of spores not activated	activation (%)	average activation (%)
Aspergillus amstelodami VTT D-76035	1	107	0	107	0	0
	2 "	103	0	103	0	
	3	108	0	108	0	

Table 1 clearly shows that the procedure described by Gyllang et al. (1977) does not result in any detectable activated spores. In contrast, treatment of *Rhizopus oryzae* ATCC 9363 spores as described in the current patent application resulted in a high level of activation of the spores as more than 90% of the spores had a size of more than 9.6  $\mu\text{m}$  and/or had one or more germ tubes per spore.

7. Conclusions. Culture homogenates of *Rhizopus oryzae* ATCC 9363, *Aspergillus fumigatus* CBS 148.89 and *Aspergillus amstelodami* VTT D-76035 prepared according to Gyllang et al. (1977) do not contain activated spores.

This experiment shows that successful activation depends on incubation of dormant spores for a sufficient time at a suitable temperature and in a suitable medium. In the spore suspension as prepared by Gyllang et al. (1977), the medium is an exhausted growth medium that does not provide the suitable conditions for spore activation, and the spores are not incubated for a sufficient time at a sufficient temperature.

8. Abbreviations used. PDA, Potato Dextrose Agar; ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures; VTT, Technical Research Centre of Finland; Ac, activated.

9. References.

- Gyllang, H., Sätmark, L. and Martinson, E., The influence of some fungi on malt quality, *EBC. Proceedings of the 16<sup>th</sup> Congress*, 1977.
- Kaiser, C., Michaelis, S. and Michell, A., *Methods in yeast genetics*, Appendix A, p. 207, Cold Spring Harbor Laboratory Press, New York, USA, 1994.
- Pitt, J.I. and Hocking, A.D. Fungi and food spoilage, second edition, Blackie Academic & Professional, London, UK, 1997.

The undersigned, being warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. §1001) and may jeopardize the validity of the application or any patent issuing thereon, hereby declares that the above statements made of my own knowledge are true and that all statements made on information and belief are believed to be true.

Date: \_\_\_\_\_  
Theo Coppens

The results of the analysis of spore activation in the culture homogenates immediately after homogenization (0 time) under the procedure of Gyllang et al. (1977) are presented in Table I.

Table I. Spore Activation at 0 Time in the Culture Homogenates.

	<u>% spores activated</u>
<i>Rhizopus oryzae</i> ATCC 9363	0
<i>Aspergillus fumigatus</i> CBS 148.89	0
<i>Aspergillus amstelodami</i> VTT D-76035	0

Further activation of spores in the culture homogenates was analyzed after 6 hours incubation of the culture homogenates at 20°C or 42°C, although this deviates from the procedure of Gyllang et al. (1977). In this procedure no incubation period is prescribed between preparation of the homogenate and inoculation of the barley. The results are presented in Table II.

Table II. Spore Activation After 6 Hours Incubation in the Culture Homogenates.

	<u>% spores activated</u>	
	Incubation at 20°C	Incubation at 42°C
<i>Rhizopus oryzae</i> ATCC 9363	0	0

<i>Aspergillus fumigatus</i> CBS 148.89	0	0
<i>Aspergillus amstelodami</i> VTT D-76035	3	0

In contrast, treatment of *Rhizopus oryzae* ATCC 9363 spores as described in the current patent application resulted in a high level of activation of the spore as more than 90% of the spores had a size of more than 9.6  $\mu\text{m}$  and/or had one or more germ tubes per spore.

7. Conclusions. Culture homogenates of *Rhizopus oryzae* ATCC 9363, *Aspergillus fumigatus* CBS 148.89 and *Aspergillus amstelodami* VTT D-76035 prepared according to Gyllang et al. (1977) do not contain activated spores. This experiment shows that successful activation depends on incubation of dormant spores for a sufficient time at a suitable temperature and in a suitable medium. In the spore suspension as prepared by Gyllang et al. (1977) the medium is an exhausted growth medium that does not provide the suitable conditions for spore activation, and the spores are not incubated for a sufficient time at a suitable temperature.

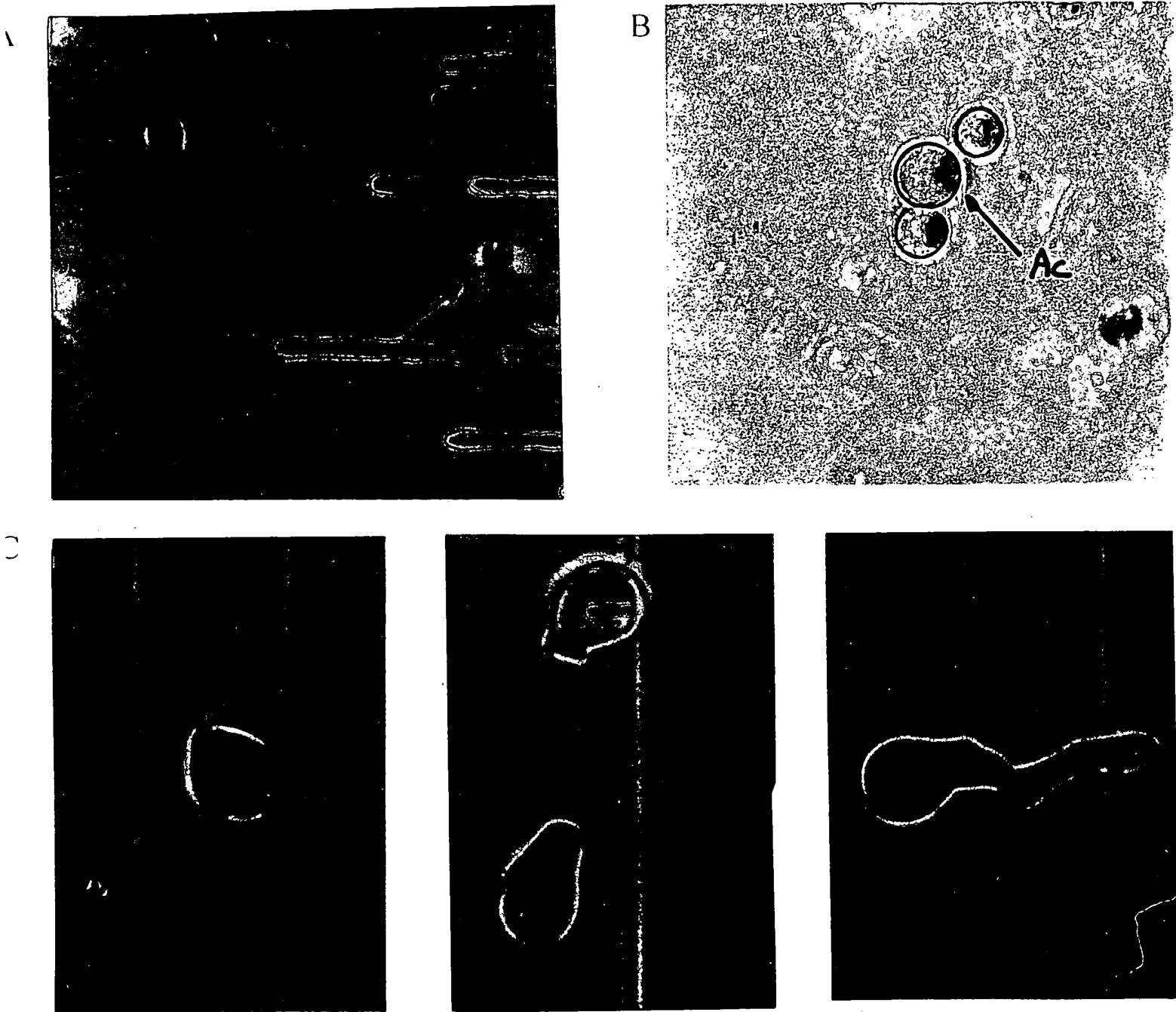
8. Abbreviation used. PDA, Potato Dextrose Agar; ATCC; American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures; VTT, Technical Research Centre of Finland; Ac, activated.

9. References.

Gyllang, H., Satmark, L. and Martinson, E., The influence of some fungi on malt quality, EBC Proceedings of the 16<sup>th</sup> Congress, 1977.

Kaiser, C., Michaelis, S. and Michell, A., Methods in yeast genetics, Appendix A, p. 207, Cold Spring Harbor Laboratory Press, New York, USA, 1994.

Pitt, J.I. and Hocking, A.D. Fungi and food spoilage, second edition, Blackie Academic & Professional, London, UK, 1997.



**Figure 1**      *Activated spores obtained by treatment as described in the current paper and dormant spores (magnification 720x): A. dormant spores; B. swollen spores with o (Ac) spore i.e. significantly more swollen than the dormant size; C. activated spores significantly more swollen than the dormant size and having one or more germ tubes per spore.*